Pre-exercise Galactose and Glucose Ingestion on Fuel Use during Exercise

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Abstract

Purpose: This study determined the effect of ingesting galactose and glucose 30 minutes prior to exercise on exogenous and endogenous fuel use during exercise. **Methods:** Nine trained male cyclists completed three bouts of cycling at 60% W_{max} for 120 minutes, after an overnight fast. Thirty minutes before exercise the cyclists ingested a fluid formulation containing placebo, 75g of galactose (Gal) or 75g of glucose (Glu) to which ^{13}C tracers had been added, in a double blind randomized manner. Indirect calorimetry and isotope ratio mass spectrometry were used to calculate fat oxidation, total carbohydrate (CHO) oxidation, exogenous CHO oxidation, plasma glucose oxidation and endogenous liver and muscle CHO oxidation rates. **Results:** Peak exogenous CHO oxidation was significantly higher following Glu (0.68 ± 0.08 g·min^{-1}, P<0.05) compared to Gal (0.44 ± 0.02 g·min^{-1}), however mean rates were not significantly different (0.40 ± 0.03 vs. 0.36 ± 0.02 g·min^{-1}, respectively). Glu produced significantly higher exogenous CHO oxidation rates during the initial hour of exercise (P<0.01), while glucose rates derived from Gal were significantly higher during the last hour (P<0.01). Plasma glucose and liver glucose oxidation at 60 minutes of exercise were significantly higher for Glu (1.07 ± 0.1 g·min^{-1}, P<0.05 and 0.57 ± 0.08 g·min^{-1}, P<0.01) compared with Gal (0.64 ± 0.05 g·min^{-1} and 0.29 ± 0.03 g·min^{-1}, respectively). There were no significant differences in total CHO, whole body endogenous CHO, muscle glycogen or fat oxidation between conditions. **Conclusion:** The pre-exercise consumption of Glu provides a higher exogenous source of CHO during the initial stages of exercise, but Gal provides the predominant exogenous source of fuel during the latter stages of exercise and reduces the reliance on liver glucose.

Carbon isotope; exogenous oxidation; liver glycogen, muscle glycogen, plasma glucose oxidation.

Paragraph 1. The consumption of carbohydrate (CHO) during exercise can enhance endurance performance (9, 20) by maintaining high plasma glucose concentrations and a high rate of CHO oxidation, especially late in exercise when muscle and liver glycogen concentrations are becoming depleted (9). Plasma glucose, as well as muscle and liver glycogen are essential for prolonged strenuous exercise (8, 16, 19). Generally the consumption of CHO during cycling exercise does not spare muscle glycogen (23, 38) but reduces the reliance upon endogenous CHO stored in the liver (23, 27).

Paragraph 2. Fuel use due to an exogenous source of CHO is dependent upon the type (s) of CHO consumed (1, 6, 42), the dose provided (23, 38, 43), as well as the timing of ingestion (7). For example, the ingestion of highly insulinogenic glucose within the hour before exercise, rather than during exercise may have a different effect on fuel use than low glycaemic index CHOs, such as fructose or galactose, which do not have a primary insulin drive (37). The extent to which this may lead to differences in fuel selection is yet to be determined.

Paragraph 3. Fructose is slowly absorbed from the intestine (18) and thus has been associated with gastro-intestinal discomfort, whereas galactose is absorbed more rapidly, at rates similar to glucose by the same sodium co-transport system (SGLT1 (41, 46)). However, glucose and galactose have uniquely different metabolic processing in the liver and peripheral tissues. Glucose provides a more immediate energy source as it can pass through the liver unchanged and enter the muscle for use and/or glycogen synthesis for subsequent use. Absorbed glucose potentially can also be taken up by the liver on the first pass (23). In contrast, galactose is converted by the liver through the Leloir pathway (17) for release as glucose, or stored as glycogen for subsequent release.

Paragraph 4. In only two studies has an evaluation of galactose during exercise using ^{13}C tracer techniques been made (6, 25). Exogenous oxidation rates of glucose derived from galactose were 48% and 61% lower in comparison to glucose during 120 minutes of cycling at ~75% and 65% of maximal oxygen uptake ($\dot{V}O_{2\,max}$), respectively. Galactose has been shown to produce significantly greater whole body endogenous CHO oxidation in comparison to glucose (124.4 ± 6.7 and 100.1 ± 3.6g (25)), which is not a consistent finding in the literature (6). Differences between the studies (6, 25) may be related to the amount of CHO provided (100g vs. 150g, respectively) because the rates of intake at the gastro-intestinal tract were similar (~1.25 $g.min^{-1}$), the prescribed relative exercise intensities and the equations used to establish exogenous oxidation rates ([29] vs. [32], respectively). Furthermore, neither study distinguished the differences between liver and muscle glycogen released during exercise. If galactose was consumed within the hour prior to exercise rather than during exercise, there may be the potential to pre-load newly synthesized liver glycogen, which may contribute to sustain plasma glucose concentrations and thus CHO oxidation during prolonged endurance exercise.

Paragraph 5. The purpose of the present study was to compare the effects of the pre-exercise ingestion (30 minutes prior to exercise) of galactose and glucose on fuel use during 120 minutes of moderate intensity cycling exercise. The use of indirect calorimetry combined with ^{13}C tracer techniques enabled the estimation of the contributions to CHO oxidation from different substrate sources. Higher galactose exogenous oxidation rates can be achieved during a second bout of exercise (25) and ~30 minutes rest is sufficient time for galactose to be converted to glucose (2). Therefore, we hypothesized that an initial bolus of galactose 30 minutes prior to exercise would produce higher exogenous oxidation rates than previously reported during an initial bout of

exercise. In addition, we hypothesized that the pre-exercise ingestion of galactose would reduce the reliance on pre-existing liver glycogen more effectively than glucose ingestion.

Methods

Participants

Paragraph 6. Nine trained male cyclists, aged: 33.1 ± 8.5 years, with a body mass of 80.3 ± 3.9 kg, $\dot{V}O_{2\,max}$ of 60.4 ± 8.0 $mL \cdot kg^{-1} \cdot min^{-1}$ and maximal power output (W_{max}) of 385.4 ± 32.5 W participated in this study. The inclusion criteria required the cyclists to have trained for ≥ 15-h per week, for at least the last 3 years. Procedures and potential risks were explained to each participant prior to the study, which was approved by the Leeds Metropolitan University ethics committee and all participants provided written informed consent.

Preliminary Testing

Paragraph 7. Participants completed a maximal incremental cycle test to volitional exhaustion to determine their individual W_{max} (24), at least 1 week before the first experimental trial on an SRM high performance ergometer (SRM, Germany). Participants cycled at an initial intensity of 100 W for 5 minutes, after which the workload was increased by 50 W every 2.5 minutes until a heart rate of 160 beats per minute, after which the work load increased by 25 W every 2.5 minutes to volitional exhaustion. W_{max} was calculated from $W_{max} = W_{out} + (t/150)$ x 25 W in which W_{out} is the highest power output (W) that the participant completed, and *t* the number of seconds the final uncompleted power output was sustained (24). W_{max} was used to determine the relative exercise intensities to be undertaken by each participant during the experimental trials (viz. power output (W) at a given % W_{max}).

Experimental Design

Paragraph 8. Participants completed three experimental cycle trials for 120 minutes at 60% W_{max}, each test separated by 7 days. Each trial involved the ingestion (30 minutes before exercise) of either 75g of galactose (Gal, (D-galactose, Hollandche, Melk & Suiker, Fabrique, The Netherlands)) or 75g of glucose (Glu, (D-glucose, Thornton and Ross Ltd, Huddersfield, UK)) or a placebo (water), as 1 litre formulations, using a randomized double blind experimental design. All formulations contained 26 $mmol \cdot L^{-1}$ sodium chloride, as well as sweetener and flavouring to blind the participants to each condition. Stock glucose (natural $\delta^{13}C$ abundance = -26.49 ‰) and galactose (natural $\delta^{13}C$ abundance = -25.96 ‰), were enriched using 0.15g and 0.75g of (D-$^{13}C_6$) glucose and (D-1-$^{13}C_1$) galactose (Sigma Aldrich, St Louis. MO, USA), as accepted (6, 25), achieving final $\delta^{13}C$ enrichments of +172.89 and +113.40 ‰, respectively. All $\delta^{13}C$ measurements are quoted with reference to the internationally accepted standard for carbon isotope measurements, Vienna Pee Dee Belemnite (VPDB). The ^{13}C abundance of stock glucose and galactose and ^{13}C enrichment of spiked glucose and galactose was determined using liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS; Isoprime, Cheadle, UK), using L-Fucose as an isotopic internal standard as previously described (29).

Diet and physical activity before testing.

Paragraph 9. Participants recorded their food intake and activity patterns during the 72-h prior to the first experimental test and were instructed to repeat the same diet and activity pattern in the 72-h before trials 2 and 3. Participants were required to refrain from any intense and/or prolonged physical activity, alcohol or caffeine consumption in the 36-h prior to each experimental trial. In addition, they were asked to refrain from ingesting CHO from plants with the C_4 photosynthetic cycle, in which natural enrichment of ^{13}C into synthesized CHO occurs

(e.g. corn, sugar cane (28)). This precaution ensured that background ^{13}C enrichment of expired CO_2 from endogenous substrate stores is less likely to be perturbed by unintentional but natural fluctuations of dietary ^{13}C. Before each test, the evening meal was standardized and taken between 6.00 pm and 8.00 pm (total 1443 kcal; 53% CHO, 18% fat, 30% protein).

Experimental Trials

Paragraph 10. Following a 12-h overnight fast participants started their experimental trials at the same time of day (between 6 am and 9am) to avoid any influence of circadian variance. Upon arrival at the laboratory, a catheter was inserted into an antecubital vein for regular blood sampling. Resting blood samples were drawn for the analysis of plasma glucose, serum insulin and plasma lactate concentrations. Resting oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) measurements were made using an online gas analysis system (Metalyser, Cortex, Germany). The digital tripleV volume transducer was calibrated using a 3-litre syringe (Hans Rudolph Inc, USA) and the gas analyzers calibrated using room air and a mass standard gas mixture (Alpha Gravimetric standard, BOC gases, Guildford, UK) of oxygen and carbon dioxide in nitrogen equivalent to expired air (15% O_2 and 5% CO_2). The test-retest reliability for $\dot{V}O_2$ and $\dot{V}CO_2$ ($l.min^{-1}$) had coefficients of variation of 1.3% and 2.4%, respectively. For the measurement of $^{13}C/^{12}C$ in expired air, 12 ml samples of expired gas were collected in duplicate in Labco Exetainers® (supplied by SerCon Ltd, Crewe, UK) via a mixing chamber (Jaeger, Germany).

Paragraph 11. Thirty minutes prior to exercise, participants consumed 1 litre of one of the three different formulations (Gal (426 $mosm \cdot kg^{-1} \cdot H_20$), Glu (423 $mosm \cdot kg^{-1} \cdot H_20$) or placebo) within 10 minutes. Participants then completed 120 minutes of cycling at 60% W_{max} on an SRM high performance ergometer (SRM, Germany). Expired air breath samples were collected and

measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ were made every 15 minutes post fluid consumption until the end of exercise. Samples of $^{13}C/^{12}C$ in expired air were collected during the final 60 seconds of each expired air collection period. Samples for the analysis of plasma glucose and serum insulin concentration were drawn at 10 minutes intervals until 60 minutes into exercise and then every 30 minutes thereafter. Samples for the analysis of plasma lactate concentration were drawn every 30 minutes and those for $^{13}C/^{12}C$ plasma glucose were drawn at 90 and 150 minutes.

Analyses

Paragraph 12. Aliquots of plasma and serum prepared by centrifugation were analyzed for selected metabolites. Glucose (Glucose Oxidase kit, Siemens Healthcare Diagnostics Inc, New York, USA), and lactate (Lactate kit, Siemens), were analyzed enzymatically using a semiautomatic analyzer (ADVIA Centaur® System, Bayer Diagnostics, Newbury, Berks, UK), whereas insulin was analyzed using an antibody assay (Insulin IRI kit, Siemens) using the same analyzer.

Paragraph 13. The $^{13}C/^{12}C$ ratio in expired air was determined through the use of isotope ratio mass spectrometry (IRMS; AP2003, GVI Instruments Ltd, Manchester, UK). The isotopic ratio $^{13}C/^{12}C$ is derived against laboratory CO_2 (itself calibrated against VPDB) from the ion beam area ratio measurements with correction of the small contribution of $^{12}C^{16}O^{17}O$ at m/z 45; the Craig correction (11). The $\delta^{13}C$ in plasma glucose and galactose, as well as galactose concentration were determined using LC-IRMS as described in detail previously (29).

Paragraph 14. Oxidation rates of total fat, total CHO, endogenous CHO (liver and muscle), plasma glucose and exogenous glucose derived from Gal and Glu ingestion, were calculated by indirect calorimetry ($\dot{V}O_2$ and $\dot{V}CO_2$) and stable isotope measurements ($^{13}C/^{12}C$ ratio in expired air and plasma), as detailed below.

Calculations

Paragraph 15. Total CHO and fat oxidation ($g \cdot min^{-1}$) were computed from $\dot{V}O_2$ ($l \cdot min^{-1}$) and $\dot{V}CO_2$ ($l \cdot min^{-1}$) using the stoichiometric equations of Péronnet & Massicotte (32), with the assumption that protein oxidation during exercise was negligible.

$$\text{Glucose } (g \cdot min^{-1}) = 4.585\,\dot{V}CO_2 - 3.226\,\dot{V}O_2 \quad (1)$$

$$\text{Lipids } (g \cdot min^{-1}) = 1.695\,\dot{V}O_2 - 1.701\,\dot{V}CO_2 \quad (2)$$

The isotopic enrichment of Glu and Gal, (R_{exo}), was expressed in standard $\delta^{13}C$ units (‰) relative to VPDB (10). Exogenous glucose oxidation derived from Gal and Glu (G_{exo}, grams) ingestion was computed by using the following equation (33), with the placebo condition establishing the background ratio of $^{13}C/^{12}C$ in expired CO_2 during exercise.

$$\text{Exogenous CHO Oxidation } (g \cdot min^{-1}) = \dot{V}CO_2\,[(R_{exp} - R_{refl})/(R_{exo} - R_{refl})]\,/k \quad (3)$$

where $\dot{V}CO_2$ is in litres per minute, R_{exp} is the observed $^{13}C/^{12}C$ in expired CO_2, R_{refl} is the $^{13}C/^{12}C$ of expired CO_2 in response to exercise when the placebo was ingested, R_{exo} is the $^{13}C/^{12}C$ of the exogenous Gal and Glu ingested, and k (0.7426 $l \cdot g^{-1}$) is the rate adjusted value for the complete oxidation of glucose (33). Endogenous CHO oxidation was calculated by subtracting exogenous oxidation from total CHO oxidation.

Paragraph 16. Computations were made on the assumption that, in response to exercise, ^{13}C is not irreversibly lost in pools of tricarboxylic acid cycle intermediates and/or bicarbonate, and that $^{13}CO_2$ recovery in expired gases was complete or almost complete during exercise (40).

Such computation has been shown to underestimate exogenous oxidation rates at the beginning of exercise because of the delay between $^{13}CO_2$ production in tissues and at the mouth (31). Using the CO_2 kinetics model proposed by Winchell et al. (45), the CO_2 exchange in the bicarbonate pool was computed. These data indicated equilibrium within the bicarbonate pool from ~15 minutes onwards for both trials. Based on this, exogenous CHO oxidation rates are presented from 15 minutes onwards during the exercise period.

Paragraph 17. On the basis of the isotopic compositions of plasma glucose (R_{glu}) the oxidation rate of plasma glucose was computed at 60 and at 120 minutes during exercise. This was modified from Peronnet et al. (34), to include placebo during exercise as the $^{13}C/^{12}C$ plasma background reference, as there were observed differences in these data at rest and during exercise.

$$\text{Plasma glucose oxidation } (g \cdot min^{-1}) = \dot{V}CO_2 \left[(R_{exp} - R_{ref1})/ (R_{glu} - R_{ref2})\right] / k \qquad (4)$$

where R_{ref2} is the isotopic composition of plasma glucose observed during exercise when the placebo was ingested. The oxidation rate of muscle glycogen ($g \cdot min^{-1}$), either directly or through the lactate shuttle (4), was calculated by subtracting plasma glucose oxidation from total CHO oxidation. Finally, the amount of glucose released from the liver was estimated as the difference between plasma glucose (equation 4) and exogenous glucose oxidation (equation 3) (34).

Statistical Analysis

Paragraph 18. Data were approximately normally distributed (Kolmogorov-Smirnov test) and are presented as mean ± SE. Two-way ANOVA for repeated measures was used to compare differences in blood related parameters and fuel use over time and between conditions. One-way ANOVA was used to compare difference in fuel use between conditions. Where significance was

detected *post hoc* analysis was performed using a paired t-test, with Bonferroni adjustment. Data were evaluated using SPSS for Windows version 17 (Chicago, USA). A 0.95 level of confidence was predetermined to denote statistical significance ($P < 0.05$).

Results

Stable Isotope Measurements

Paragraph 19. The $^{13}C/^{12}C$ in expired CO_2 was significantly higher for Glu from the start of exercise until 90 minutes (range: -20.68 to +5.32 ‰, $P < 0.01$) in comparison to Gal (range: -23.47 to +13.53 ‰), with Gal having significantly higher $^{13}C/^{12}C$ in expired CO_2 compared to Glu at 135 minutes (-11.58 ± 0.54 vs. -16.21 ± 0.53 ‰, $P < 0.001$) and 150 minutes (-12.79 ± 0.46 vs. -18.72 ± 0.50 ‰, $P < 0.001$), Fig. 1A. During the placebo condition, there was a small but significant increase (average difference: 1.02 ‰, $P < 0.01$) in $^{13}C/^{12}C$ in expired CO_2 over time, from 105 minutes onwards in comparison to resting breath samples. These data were used as a background correction for the calculation of exogenous oxidation for the Gal and Glu conditions.

Exogenous and Endogenous CHO Oxidation

Paragraph 20. Exogenous CHO oxidation rates reached 0.66 ± 0.08 $g \cdot min^{-1}$ during the initial 15 minutes of exercise for Glu, which was significantly higher ($P < 0.001$) than glucose rates derived from Gal (0.27 ± 0.02 $g \cdot min^{-1}$), Fig 1, B. The exogenous oxidation of Glu then decreased, while the oxidation rates of glucose derived from Gal steadily increased, with a crossover point occurring between the two conditions between 90 and 105 minutes. Exogenous CHO oxidation rates were significantly higher ($P < 0.01$) throughout exercise until 90 minutes

for Glu in comparison to Gal. From 105 minutes glucose oxidation rates derived from Gal were significantly higher ($P < 0.01$), peaking at 120 minutes (0.44 ± 0.02 g·min^{-1}).

Paragraph 21. There was inter-individual variability in the time taken to reach peak exogenous CHO oxidation rates. Therefore, the above data does not truly reflect the differences in peak exogenous oxidation rates achieved for each condition. The mean of each of the individual's peak exogenous CHO oxidation rate was significantly higher ($P < 0.05$) for Glu (0.68 ± 0.08 g·min^{-1}) in comparison to Gal (0.44 ± 0.02 g·min^{-1}), with individual CHO oxidation peaks occurring at mean time points of 60 and 150 min (30 and 120 minutes into exercise), respectively. The average exogenous CHO oxidation rates for Glu (0.40 ± 0.03 g·min^{-1}) were not significantly different over the 120 minutes of exercise in comparison to Gal (0.36 ± 0.02 g·min^{-1}). The relative contribution of exogenous CHO to total energy expenditure for the 120 minutes of exercise was also significantly higher ($P < 0.01$) for Glu ($8.67 \pm 0.54\%$) compared to Gal ($7.06 \pm 0.33\%$) as shown in Fig 2. In addition, the oxidation efficiency was significantly ($P < 0.01$) higher for Glu ($71.07 \pm 5.91\%$) compared to Gal ($53.16 \pm 3.13\%$).

Paragraph 22. Endogenous CHO oxidation rates increased during the initial 15 minutes of exercise achieving average oxidation rates of 2.82 ± 0.23 g·min^{-1}, 2.81 ± 0.38 g·min^{-1} and 2.87 ± 0.37 g·min^{-1} for Gal, Glu and placebo conditions, respectively, over the 120 minutes of exercise (Fig 3, B). There were no significant differences between any of the conditions. There were also no significant differences between conditions in the relative contributions of endogenous CHO oxidation to the total energy expenditure over the 120 minutes of exercise (Fig 2).

Total CHO and Fat Oxidation

Paragraph 23. Total CHO oxidation peaked 15 minutes into exercise for Gal and Glu (on average 3.48 ± 0.31 g·min^{-1}), whereas following placebo there was a delayed response, with peak

values achieved at 30 minutes into exercise (2.97 ± 0.34 g·min^{-1}), with relatively stable CHO oxidation rates thereafter (Fig 3, A). During the placebo condition the CHO oxidation rates were consistently lower in comparison to Glu and Gal, but there were no significant interactions between conditions.

Paragraph 24. Fat oxidation rates (Table 1) progressively increased throughout exercise for all three conditions, with average rates of 0.64 ± 0.04, 0.59 ± 0.10 and 0.72 ± 0.10 g·min^{-1} for the 120 minutes of exercise, for Gal, Glu and placebo, respectively. There were no significant interactions between conditions. The relative contribution of fat to total energy expenditure during the 120 minutes of exercise is shown in Fig 2.

Oxidation of Plasma Glucose, Liver Glucose and Muscle Glycogen

Paragraph 25. The rate of plasma glucose oxidation derived from Gal was significantly ($P < 0.01$) greater at 120 minutes compared to 60 minutes (1.29 ± 0.16 and 0.64 ± 0.05 g·min^{-1}, respectively), Fig 4. This was due to an increase in exogenous CHO oxidation and a significant increase in liver glucose oxidation rates from 60 to 120 minutes for Gal ($P < 0.01$). In contrast, there was no significant change in plasma glucose oxidation for the Glu condition (1.07 ± 0.11 and 1.22 ± 0.15 g·min^{-1}), as the significant ($P < 0.001$) decrease in exogenous CHO oxidation was accompanied by a significant increase (48%, $P < 0.01$) in liver glucose oxidation. Plasma, liver and exogenous CHO oxidation were significantly ($P < 0.01$) greater for Glu at 60 minutes compared to Gal. There were no significant differences in muscle glycogen oxidation rates at 60 and 120 minutes between Gal and Glu conditions. However, the muscle glycogen oxidation rate significantly ($P < 0.01$) decreased from 60 to 120 minutes for Gal (2.5 ± 0.25 and 1.81 ± 0.27 g·min^{-1}, respectively) and Glu (2.18 ± 0.39 and 1.93 ± 0.38 g·min^{-1}, respectively).

Blood Biochemistry

Paragraph 26. Plasma glucose and serum insulin concentrations following the pre-exercise ingestion of Glu increased to peaks of 7.4 ± 0.6 mmol·L^{-1} (Fig. 5A) and 36.3 ± 7.5 μU·mL^{-1} (Fig. 5B), respectively, directly prior to exercise. These were significantly higher in comparison to placebo (4.8 ± 0.1 mmol·L^{-1} and 4.5 ± 1.1 μU·mL^{-1} ($P < 0.05$), respectively) and Gal conditions (4.9 ± 0.3 mmol·L^{-1} ^{1} and 9.1 ± 2.0 μU·mL^{-1} ($P < 0.05$), respectively).

Paragraph 27. After the onset of exercise, mean plasma glucose concentrations fell rapidly to nadirs of 3.44 ± 0.28 mmol·L^{-1} and 3.59 ± 0.20 mmol·L^{-1}, for Glu and Gal, respectively, during the initial 20 minutes of exercise. Mean plasma glucose concentrations for placebo remained stable ((4.71 ± 0.15 mmol·L^{-1} (40 minute time point), 5.01 ± 0.12 mmol·L^{-1} (50 minute time point)) and were significantly higher ($P < 0.05$) than the Glu and Gal conditions at 50 minutes. After the first 20 minutes of exercise (50 min from ingestion) mean plasma glucose concentrations increased back to basal concentrations by 70 minutes for Glu (4.83 ± 0.18 mmol·L^{-1}) and Gal (4.93 ± 0.11 mmol·L^{-1}), with relative stability in plasma glucose concentrations thereafter. Mean plasma glucose concentrations were also significantly higher for Gal and placebo at 60 minutes ($P < 0.05$) compared to Glu. During the last hour of exercise plasma glucose concentrations for all three conditions started to slowly decrease below basal concentrations, with no significant differences between the three conditions. After the onset of exercise mean serum insulin concentrations took longer to decrease back towards baseline values for Glu (3.43 ± 0.74 μU·mL^{-1}, 60 minutes) compared to Gal (2.98 ± 0.40 μU·mL^{-1}, 40 minutes). Once fasting concentrations had been reached for each of the three conditions, concentrations remained stable until the end of exercise.

Paragraph 28. Plasma galactose concentrations decreased from 4.88 ± 0.50 mmol·L^{-1} at 60 minutes into exercise to 0.91 ± 0.09 mmol·L^{-1} at the end of the 120 minutes of exercise, following Gal ingestion.

Paragraph 29. Plasma lactate concentrations increased from resting values of 0.81 mmol·L^{-1} to approximately 1.63 mmol·L^{-1} after the initial 30 minutes of exercise, but increased to approximately 1.95 mmol·L^{-1} after 120 minutes of exercise. There was no significant difference in responses between the three conditions.

Discussion

Paragraph 30. This is the first study to evaluate the effects of the pre-exercise ingestion of galactose on plasma glucose oxidation and endogenous glucose contributions from the oxidation of glucose released from the liver and muscle glycogen oxidation (including the lactate shuttle) from pre-existing glycogen. The primary findings are that galactose ingestion reduces the reliance on liver glucose (from pre-exiting glycogen) during exercise and provides a more progressive energy source, with significantly higher oxidation of glucose derived from exogenous galactose during the last hour of exercise in comparison to exogenous glucose. In contrast, glucose ingestion produced higher exogenous oxidation rates during the initial hour of exercise, with greater availability in plasma glucose oxidation with a trend for a decreased reliance on muscle glycogen oxidation (though not significant) midway through exercise.

Paragraph 31. The peak exogenous CHO oxidation rate (defined as the mean of the highest value reached by each individual) of glucose derived from Gal (0.44 ± 0.02 g·min^{-1}) was lower than Glu (0.68 ± 0.08 g·min^{-1}). However, the peak exogenous CHO oxidation rates following galactose ingestion are to the authors' knowledge, the highest reported in the literature to date

during an initial bout of exercise. Leijssen et al. (25) using the equation of Mosora et al. (30) reported a peak exogenous oxidation rate of 0.41 g·min^{-1} during an initial bout of exercise. Caution in the interpretation of this value is required, due to difficulties associated with low isotopic enrichment and lack of $^{13}C/^{12}C$ ratios during exercise from a placebo trial. This equation may overestimate exogenous oxidation rates, as using the $^{13}C/^{12}C$ ratio at rest rather than during exercise is not a true reflection of substrate metabolism during exercise (33). For these reasons the present study used a high isotopic enrichment (+113.4 ‰) and the ^{13}C abundance measured during the placebo trial was used for corrections (33). Further, the consumption of galactose 30 minutes prior to exercise, may have enabled the liver to process galactose to glucose and liver glycogen more effectively, enabling greater peak exogenous CHO oxidation rates than the provision of repeated dosages throughout exercise (6, 25).

Paragraph 32. The mean exogenous CHO oxidation rate of glucose derived from Gal (0.36 ± 0.02 g·min^{-1}) is higher compared to other studies (0.24 g·min^{-1} (6), 0.27 g·min^{-1}, (25)). The timing and frequency of ingestion may be an explanation for the differences, as these studies used multiple doses throughout exercise (6, 25), rather than a single bolus before exercise. In addition, the lower relative exercise intensity used by Burrelle et al. (6) in comparison to the present study (65% vs. 70% $\dot{V}O_{2\,max}$, respectively) is another possible explanation, as exogenous CHO oxidation has been shown to increase with exercise intensity (35).

Paragraph 33. The differences in exogenous CHO oxidation rates following Gal and Glu ingestion are most likely explained by their different metabolic processing in the liver and peripheral tissues. On entering the circulation galactose is preferentially taken up by the liver (44) prior to conversion to glucose-1-phospate via the Leloir pathway (17). Glucose-1-phosphate is then available for the formation of glycogen in the liver or is released as free glucose (14). The

preferential conversion of galactose into liver glycogen is supported by previous data (12, 25). The literature (25) has showed that the exogenous CHO oxidation rate of glucose derived from galactose was much higher (0.85 $g \cdot min^{-1}$) during a second bout of exercise (30 minutes), following a 60 minute recovery period. This may reflect the release and oxidation of glucose from liver glycogen synthesised from galactose during the initial exercise period and the subsequent recovery. Furthermore, a recent ^{13}C magnetic resonance spectroscopy study (12) revealed that ingestion of maltodextrin with galactose, was twice as effective at restoring liver glycogen compared to maltodextrin plus glucose.

Paragraph 34. The metabolic fate of galactose is likely to explain the progressive increase over time in exogenous CHO oxidation following Gal ingestion. This may be a reflection of the time taken to convert galactose or glycogen recently formed from galactose, into glucose, before it can be released into the systemic circulation.

Paragraph 35. Glucose oxidation is controlled in part by plasma glucose concentrations (15). Therefore, the higher exogenous CHO oxidation rates at the initiation and for the first part of exercise during the use of Glu may be attributed to the higher plasma glucose concentrations, as well as the higher serum insulin concentrations, directly prior to exercise in comparison to Gal. The higher serum insulin concentrations combined with increased muscular contraction, increases glucose uptake and thus glucose oxidation, with increased uptake reflected in the decreasing plasma glucose concentrations at the occurrence of peak oxidation rates. The transient decline in plasma glucose concentrations from a relative hyperglycaemia (prior to exercise) reflects a change in glucose flux into the muscle, which is consistent with the hyperinsulinaemia (shown directly prior to exercise for this condition) and an effect of increased contractile activity on muscle glucose uptake (13, 26).

Paragraph 36. The present study showed that overall, significantly more of the exogenous source of Glu was oxidized (71.1 ± 5.9%) in comparison to Gal (53.2 ± 3.1%). Therefore, a significant amount of the ingested Glu (27.4 ± 4.0g) and a larger amount of Gal (35.1 ± 2.4g) remained unaccounted for. Disappearance may in part be related to gastric emptying, as a single bolus of 75g was provided, and it is likely that a proportion of the glucose or galactose may have remained in the gut. However, none of the cyclists reported any gastro-intestinal problems prior to or during the exercise period. It is unlikely that the gastric emptying (25, 36), was limited by the exercise intensity during the exercise period, which was not above 70% $\dot{V}O_{2\,max}$ (5), or the rate of uptake and oxidation by the muscle (15). There is the possibility that glucose was absorbed and taken up and retained by the liver (23), which may also be the case for galactose. For glucose only, direct uptake by muscles is also possible. Since glucose release from the liver is highly controlled and may also be rate limited (24) the disposal of both glucose and galactose into hepatic metabolism before exercise in amounts that are not all used by the subsequent exercise was likely to be a significant part of the doses not accounted for in the present study. The loss of galactose in urine (galactosuria) has also been identified (39) presumably due to its low renal threshold (0.5 $mmol \cdot L^{-1}$ (44)) and thus could account for some galactose disposal.

Paragraph 37. An original aspect of this study was the comparison of plasma glucose, liver glucose and muscle glycogen oxidation (including the lactate shuttle) rates during exercise, following galactose and glucose ingestion. These data indicate that plasma glucose oxidation rates following Glu ingestion are greater during the initial hour of exercise in comparison to Gal. Glucose is more likely to be used as an immediate energy source by muscle, especially when plasma glucose and serum insulin concentrations are still high as seen at the onset of exercise. Galactose disposal, as a source of plasma glucose for muscle oxidation, would be constrained by

the need to be supplied either from newly formed liver glycogen or from conversion by the Leloir pathway (14). However, this study was unable to report the quantity of galactose metabolized and stored as liver glycogen to be subsequently released as glucose. The lower plasma glucose oxidation derived from Gal at 60 minutes into exercise was balanced by a slightly increased muscle glycogen oxidation (including the lactate shuttle) in comparison to Glu (though not significant), as there were no significant differences in whole body endogenous CHO oxidation between conditions. In contrast, the greater availability of plasma glucose following Glu ingestion at 60 minutes into exercise showed a trend for a decreased reliance on muscle glycogen oxidation (though not significant).

Paragraph 38. This is the first study to show that glucose released from the liver (from pre-existing liver glycogen) was used to a lesser extent following Gal ingestion in comparison to Glu, with an increase in use over time for both conditions. Galactose is a precursor for liver glycogen synthesis (12, 14), which may explain the reduced reliance on liver glucose from pre-existing stores during both the initial and final parts of exercise. Even though the oxidation of glucose released from the liver (from pre-existing liver glycogen) was higher following Glu ingestion it is feasible that the associated hyperinsulineamia may have restricted hepatic output, as high insulin concentrations have been shown to inhibit hepatic output (23). The increased release of glucose from the liver over time for both conditions, to maintain effective plasma glucose oxidation, has been suggested to be related to depleting muscle glycogen concentrations (3, 34), but still there was a smaller contribution during the use of Gal. However, muscle glycogen oxidation (including the lactate shuttle) was still relatively high due to the stability in total CHO oxidation rates, which is not consistent with depleted muscle glycogen stores. It is important to note that ^{13}C glucose and ^{13}C galactose can undergo recycling and may

underestimate the rate of plasma glucose and hepatic oxidation, though is likely to be negligible during exercise (4 to 10% (22)).

Paragraph 39. Even though there are differences in exogenous CHO, endogenous liver glucose and muscle glycogen oxidation rates throughout the two hour exercise period, there was no significant effects on the absolute or relative whole body endogenous CHO oxidation rates between the Gal and Glu conditions. Furthermore, despite higher serum insulin concentrations following Glu ingestion, there were not significant differences in total fat oxidation between conditions. Whether or not the same findings would occur with the pre-exercise ingestion of galactose, following a CHO rich meal several hours prior to exercise is yet to be established.

Paragraph 40. The maintenance of an adequate plasma glucose oxidation, as well as a reduced reliance on liver glycogen and muscle glycogen would theoretically be beneficial for endurance performance (21). Therefore, there is the possibility that when CHO is prescribed pre-event glucose ingestion may produce benefits for performance due to higher rates of plasma glucose oxidation during the initial part of exercise. This is further supported by the trend to reduce reliance on muscle glycogen oxidation (including the lactate shuttle). In contrast, galactose provided a more progressive release of energy over time following its conversion to glucose, as well as reducing the reliance on glucose released from the pre-existing liver glycogen. This may be important for longer endurance activities where the maintenance of plasma glucose concentrations becomes more important, as muscle glycogen becomes depleted over time. Furthermore, significantly more of the exogenous source of Glu was oxidized in comparison to Gal, which may provide additional support for Glu in terms of endurance performance. Whether or not galactose improves performance directly, or whether the combined use of galactose and

glucose would be more beneficial for potential performance outcomes is yet to be fully established as is the combined use of galactose prior to and during exercise.

Paragraph 41. In conclusion, the present study showed that following the ingestion of Gal 30 minutes prior to exercise, peak exogenous oxidations rates of glucose derived from Gal of 0.44 ± 0.02 $g \cdot min^{-1}$ can be achieved. This supports the hypothesis, that an initial bolus of galactose 30 minutes prior to exercise would produce higher exogenous glucose oxidation rates than previously reported. However, Glu provided a more immediate energy source, whereas Gal provided a more progressive glucose oxidation response over the duration of the exercise period, as well as sparing pre-existing liver glycogen stores.

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Disclosures

The authors declare no conflict of interest.

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Figures

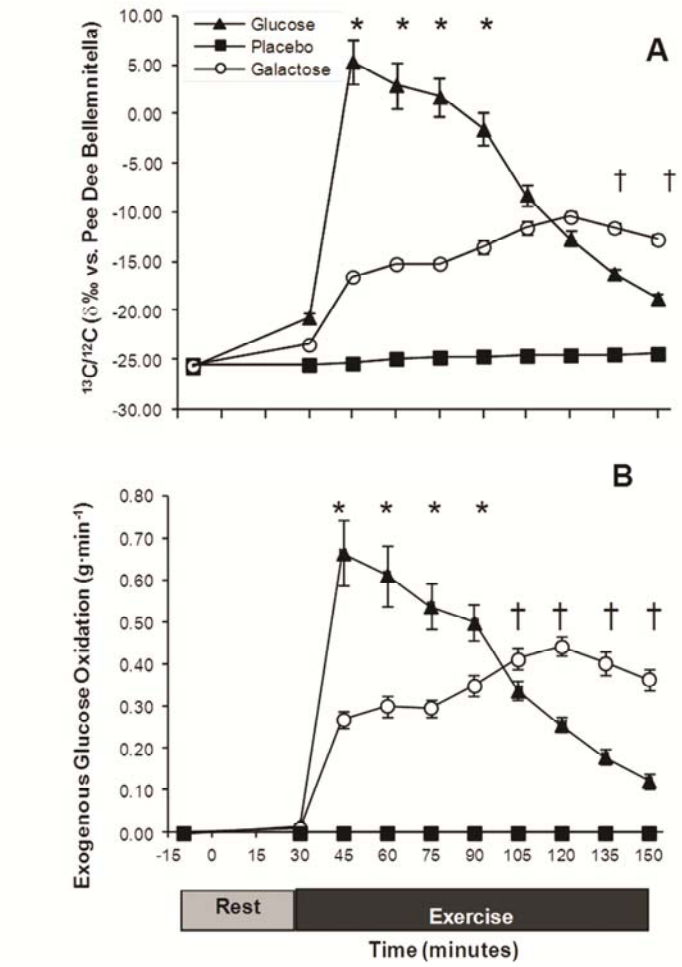

Fig 1. Changes in $^{13}C/^{12}C$ in expired CO_2 (A) and exogenous carbohydrate oxidation (B) at rest and during exercise following the pre-exercise ingestion of placebo and ^{13}C labelled glucose (Glu) and galactose (Gal). Values are mean ± SE; N=9. * Glu significantly higher than Gal ($P<0.05$). † Gal significantly higher than Glu ($P<0.05$).

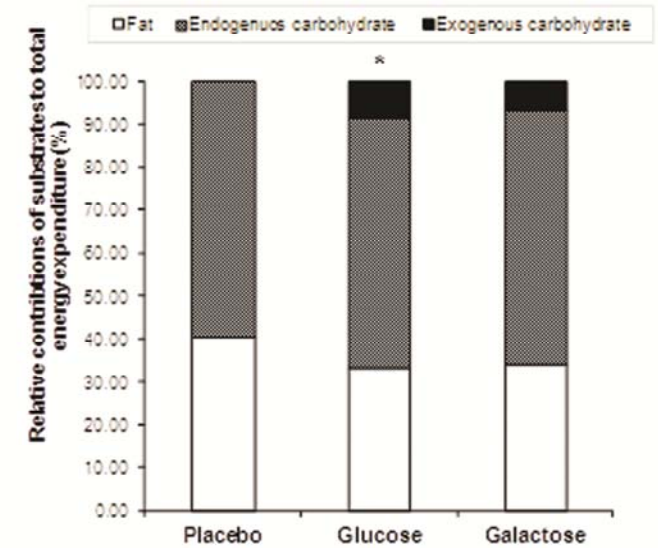

Fig 2. The relative (%) contributions of substrate oxidation to total energy expenditure for each trial during 120 minutes of exercise. * relative exogenous oxidation for Glu significantly higher than Gal ($P<0.05$).

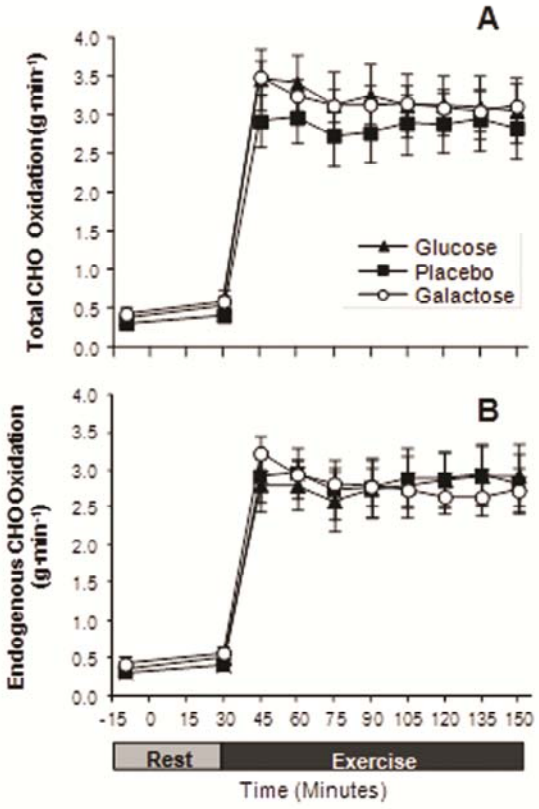

Fig 3. Total carbohydrate oxidation (A) and endogenous carbohydrate oxidation (B) at rest and during exercise following each of the three trials. Values are mean ± SE; N=9.

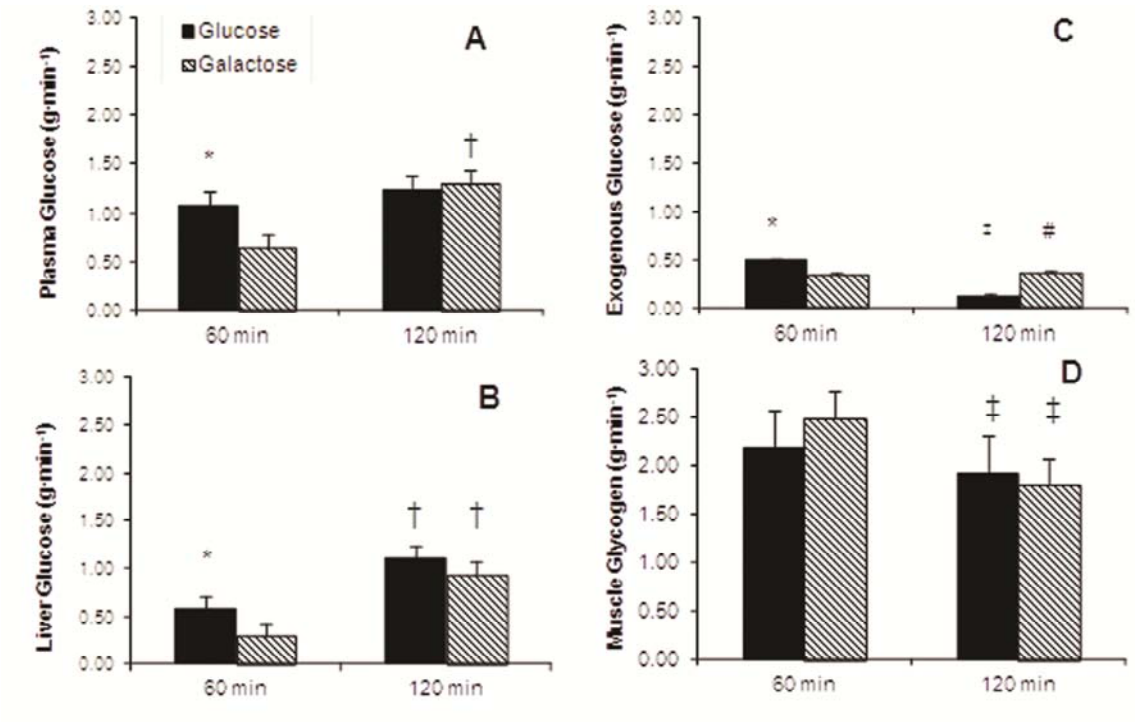

Fig 4. Oxidation rates of plasma glucose (A) glucose release from the liver (B), exogenous carbohydrate (C) and muscle glycogen (D) at 60 minutes and 120 minutes during exercise. Values are means ± SE; N=9. * Glu significantly higher than Gal at 60 minutes, $P < 0.01$. # Gal significantly higher than Glu at 120 minutes, $P < 0.01$. † 120 minutes significantly higher than 60 minutes, $P < 0.05$. ‡ 120 min significantly lower than 60 minutes, $P < 0.05$.

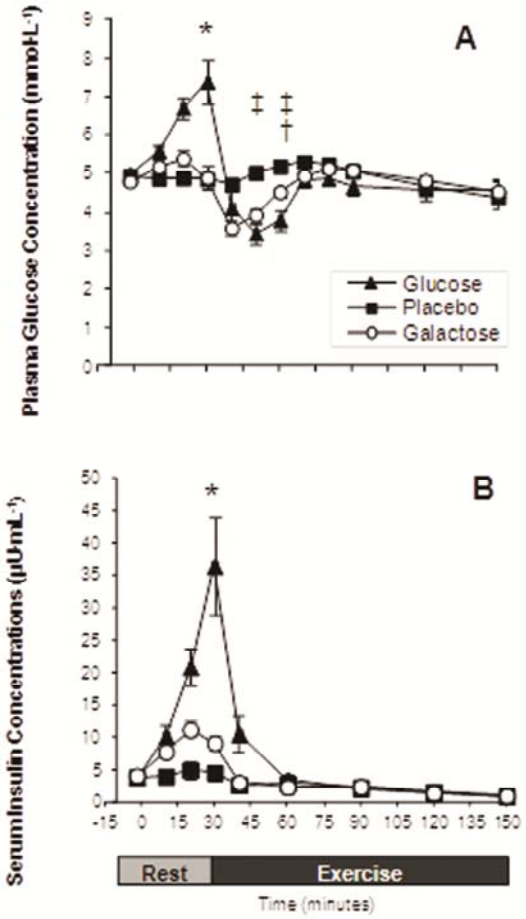

Fig 5. Plasma glucose (A) and serum insulin (B) concentrations. * Glu significantly higher than Gal and placebo ($P<0.05$). ‡ placebo significantly higher than Glu and Gal ($P<0.05$). † Gal significantly higher than Glu ($P<0.05$).

Table 1. Total fat oxidation ($g \cdot min^{-1}$) at rest and during exercise following each of the three trials

Time (minutes)	Placebo	Galactose	Glucose
-10	0.16 ± 0.04	0.11 ± 0.03	0.15 ± 0.02
30	0.13 ± 0.02	0.10 ± 0.04	0.10 ± 0.02
45	0.55 ± 0.09	0.43 ± 0.03	0.44 ± 0.09
60	0.72 ± 0.09	0.59 ± 0.04	0.50 ± 0.10
75	0.72 ± 0.12	0.66 ± 0.04	0.54 ± 0.10
90	0.76 ± 0.11	0.68 ± 0.04	0.61 ± 0.10
105	0.78 ± 0.12	0.68 ± 0.04	0.61 ± 0.10
120	0.79 ± 0.11	0.69 ± 0.04	0.68 ± 0.10
135	0.73 ± 0.10	0.70 ± 0.05	0.68 ± 0.11
150	0.71 ± 0.10	0.71 ± 0.06	0.65 ± 0.10